

***Listing of the Claims***

This listing of claims will replace all prior versions, and listings of claims in the application.

1 - 175. (Cancelled)

176. (New) A composition comprising embryonic stem cells and a serum-free cell culture medium capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

177. (New) The composition of claim 176,  
wherein the composition is capable of being stored indefinitely at less than or equal to about  $-135^{\circ}\text{C}$ , and

wherein embryonic stem cells of the composition can be cultivated after storing the composition at less than or equal to about  $-135^{\circ}\text{C}$ .

178. (New) The composition of claim 176, wherein the embryonic stem cells are obtained from a human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, or amphibian.

179. (New) The composition of claim 178, wherein the embryonic stem cells are obtained from a human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, or bird.

180. (New) The composition of claim 179, wherein the embryonic stem cells are obtained from a mouse, cow, goat, or sheep.

181. (New) The composition of claim 180, wherein the embryonic stem cells are obtained from a mouse.

182. (New) The composition of claim 179, wherein the embryonic stem cells are obtained from a human.

183. (New) The composition of claim 176, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

184. (New) The composition of claim 176, wherein the serum-free cell culture medium comprises leukemia inhibiting factor.

185. (New) The composition of claim 176, wherein the serum-free cell culture medium comprises steel factor.

186. (New) The composition of claim 176, wherein the serum-free cell culture medium comprises ciliary neurotrophic factor.

187. (New) The composition of claim 176, wherein the serum-free cell culture medium comprises oncostatin M.

188. (New) The composition of claim 176,  
wherein the serum-free cell culture medium comprises a basal cell culture medium,  
wherein the basal cell culture medium comprises a serum-free supplement, and  
wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element.

189. (New) The composition of claim 188,  
wherein the composition is capable of being stored indefinitely at less than or equal to about  $-135^{\circ}\text{C}$ , and  
wherein embryonic stem cells of the composition can be cultivated after storing the composition at less than or equal to about  $-135^{\circ}\text{C}$ .

190. (New) The composition of claim 188, wherein the embryonic stem cells are obtained from a human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, or amphibian.

191. (New) The composition of claim 190, wherein the embryonic stem cells are obtained from a human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, or bird.

192. (New) The composition of claim 191, wherein the embryonic stem cells are obtained from a mouse, cow, goat, or sheep.

193. (New) The composition of claim 192 wherein the embryonic stem cells are obtained from a mouse.

194. (New) The composition of claim 191, wherein the embryonic stem cells are obtained from a human.

195. (New) The composition of claim 188, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

196. (New) The composition of claim 188, wherein the serum-free cell culture medium comprises leukemia inhibiting factor.

197. (New) The composition of claim 188, wherein the serum-free cell culture medium comprises steel factor.

198. (New) The composition of claim 188, wherein the serum-free cell culture medium comprises ciliary neurotrophic factor.

199. (New) The composition of claim 188, wherein the serum-free cell culture medium comprises oncostatin M.

200. (New) The composition of claim 188, wherein the albumin is bovine albumin.

201. (New) The composition of claim 188, wherein the albumin is human albumin.

202. (New) The composition of claim 201, wherein the albumin is a lipid-rich.

203. (New) The composition of claim 201, wherein the albumin is a lipid-poor.

204. (New) The composition of claim 188, wherein the transferrin is bovine transferrin.

205. (New) The composition of claim 188, wherein the transferrin is human transferrin.

206. (New) The composition of claim 205, wherein the transferrin is iron-saturated.

207. (New) The composition of claim 188, wherein the insulin is bovine insulin.

208. (New) The composition of claim 188, wherein the insulin is human insulin.

209. (New) The composition of claim 188, wherein the insulin is recombinant insulin.

210. (New) A composition comprising embryonic stem cells and a serum-free cell culture medium,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

211. (New) The composition of claim 210,

wherein the composition is capable of being stored indefinitely at less than or equal to about  $-135^{\circ}\text{C}$ , and

wherein embryonic stem cells of the composition can be cultivated after storing the composition at less than or equal to about  $-135^{\circ}\text{C}$ .

212. (New) The composition of claim 210, wherein the embryonic stem cells are obtained from a human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, or amphibian.

213. (New) The composition of claim 212, wherein the embryonic stem cells are obtained from a human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, or bird.

214. (New) The composition of claim 213, wherein the embryonic stem cells are obtained from a mouse, cow, goat, or sheep.

215. (New) The composition of claim 214, wherein the embryonic stem cells are obtained from a mouse.

216. (New) The composition of claim 213 wherein the embryonic stem cells are obtained from a human.

217. (New) The composition of claim 210, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

218. (New) The composition of claim 210, wherein the serum-free cell culture medium comprises leukemia inhibiting factor.

219. (New) The composition of claim 210, wherein the serum-free cell culture medium comprises steel factor.

220. (New) The composition of claim 210, wherein the serum-free cell culture medium comprises ciliary neurotrophic factor.

221. (New) The composition of claim 210, wherein the serum-free cell culture medium comprises oncostatin M.

222. (New) The composition of claim 210, wherein the albumin is bovine albumin.

223. (New) The composition of claim 210, wherein the albumin is human albumin.

224. (New) The composition of claim 223, wherein the albumin is a lipid-rich.



225. (New) The composition of claim 223, wherein the albumin is a lipid-poor.
226. (New) The composition of claim 210, wherein the transferrin is bovine transferrin.
227. (New) The composition of claim 210, wherein the transferrin is human transferrin.
228. (New) The composition of claim 227, wherein the transferrin is iron-saturated.
229. (New) The composition of claim 210, wherein the insulin is bovine insulin.
230. (New) The composition of claim 210, wherein the insulin is human insulin.
231. (New) The composition of claim 210, wherein the insulin is recombinant insulin.
232. (New) A product of manufacture comprising a container means,  
wherein the container means contains embryonic stem cells and a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein a basal cell culture medium supplemented with the serum-free supplement is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

233. (New) The product of manufacture of claim 232 in a frozen state.

234. (New) The product of manufacture of claim 232 not containing leukemia inhibitory factor.

235. (New) The product of manufacture of claim 232 containing leukemia inhibiting factor.

236. (New) The product of manufacture of claim 232 containing steel factor.

237. (New) The product of manufacture of claim 232 containing ciliary neurotrophic factor.

238. (New) The product of manufacture of claim 232 containing oncostatin M.

239. (New) The product of manufacture of claim 232, wherein the albumin is bovine albumin.

240. (New) The product of manufacture of claim 232, wherein the albumin is human albumin.

241. (New) The product of manufacture of claim 240, wherein the albumin is a lipid-rich.

242. (New) The product of manufacture of claim 240, wherein the albumin is a lipid-poor.

243. (New) The product of manufacture of claim 232, wherein the transferrin is bovine transferrin.

244. (New) The product of manufacture of claim 232, wherein the transferrin is human transferrin.

245. (New) The product of manufacture of claim 244, wherein the transferrin is iron-saturated.

246. (New) The product of manufacture of claim 232, wherein the insulin is bovine insulin.

247. (New) The product of manufacture of claim 232, wherein the insulin is human insulin.

248. (New) The product of manufacture of claim 232, wherein the insulin is recombinant insulin.

249. (New) A product of manufacture comprising a container means,  
wherein the container means contains embryonic stem cells and a serum-free cell culture medium,

wherein the serum-free cell culture medium comprises a basal cell culture medium,  
wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

250. (New) A product of manufacture comprising a container means,

wherein the container means contains embryonic stem cells and a serum-free cell culture medium,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

251. (New) A product of manufacture comprising a first container means and a second container means,

wherein the first container means contains a serum-free supplement,

wherein the second container means contains embryonic stem cells,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein a basal cell culture medium supplemented with the serum-free supplement is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

252. (New) The product of manufacture of claim 251 further comprising a third container means containing a basal medium.

253. (New) A product of manufacture comprising a first container means and a second container means,

wherein the first container means contains a serum-free cell culture medium,

wherein the second container means contains embryonic stem cells,

wherein the serum-free cell culture medium comprises a basal cell culture medium,

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

254. (New) A product of manufacture comprising a first container means and a second container means,

wherein the first container means contains a serum-free cell culture medium,

wherein the second container means contains embryonic stem cells,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

255. (New) A method for expanding embryonic stem cells comprising contacting embryonic stem cells with a serum-free cell culture medium capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

256. (New) The method of claim 255 further comprising seeding the embryonic stem cells upon a layer of feeder cells.

257. (New) The method of claim 256, wherein the feeder cells are primary embryonic fibroblasts, inactivated feeder cells, or STO cells.

258. (New) The method of claim 255, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

259. (New) The method of claim 255, wherein the serum-free cell culture medium comprises leukemia inhibiting factor.

260. (New) The method of claim 255, wherein the serum-free cell culture medium comprises steel factor.

261. (New) The method of claim 255, wherein the serum-free cell culture medium comprises ciliary neurotrophic factor.

262. (New) The method of claim 255, wherein the serum-free cell culture medium comprises oncostatin M.

263. (New) A method for expanding embryonic stem cells in a serum-free cell culture medium comprising

- (a) contacting embryonic stem cells with a serum-free cell culture medium, and
- (b) expanding the embryonic stem cells,

wherein the serum-free cell culture medium comprises a basal cell culture medium, wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.



- 264. (New) The method of claim 263, wherein the albumin is bovine albumin.
- 265. (New) The method of claim 263, wherein the albumin is human albumin.
- 266. (New) The method of claim 265, wherein the albumin is a lipid-rich.
- 267. (New) The method of claim 265, wherein the albumin is a lipid-poor.
- 268. (New) The method of claim 263, wherein the transferrin is bovine transferrin.
- 269. (New) The method of claim 263, wherein the transferrin is human transferrin.
- 270. (New) The method of claim 269, wherein the transferrin is iron-saturated.
- 271. (New) The method of claim 263, wherein the insulin is bovine insulin.
- 272. (New) The method of claim 263, wherein the insulin is human insulin.
- 273. (New) The method of claim 263, wherein the insulin is recombinant insulin.
- 274. (New) A method for preventing the differentiation of embryonic stem cells in  
a serum-free cell culture medium comprising

(a) contacting embryonic stem cells with a serum-free cell culture medium, and  
(b) expanding the embryonic stem cells,  
wherein the serum-free cell culture medium comprises a basal cell culture medium,  
wherein the basal cell culture medium is supplemented with a serum-free  
supplement,  
wherein the serum-free supplement comprises an albumin, an albumin substitute, an  
amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an  
insulin substitute, a collagen precursor, or a trace element, and  
wherein the serum-free cell culture medium is capable of preventing differentiation  
of the embryonic stem cells during expansion of the embryonic stem cells.

275. (New) A method for preventing the differentiation of embryonic stem cells in  
a serum-free cell culture medium comprising

(a) contacting embryonic stem cells with a serum-free cell culture medium, and  
(b) expanding the embryonic stem cells,  
wherein the serum-free cell culture medium is obtained by combining a basal cell  
culture medium with a serum-free supplement,  
wherein the serum-free supplement comprises an albumin, an albumin substitute, an  
amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an  
insulin substitute, a collagen precursor, or a trace element, and  
wherein the serum-free cell culture medium is capable of preventing differentiation  
of the embryonic stem cells during expansion of the embryonic stem cells.

276. (New) A method for causing embryonic stem cells to differentiate in a serum-free cell culture medium comprising

- (a) contacting embryonic stem cells with a serum-free cell culture medium,
- (b) expanding the embryonic stem cells, and
- (c) adding a differentiation factor or changing culturing conditions thereby causing the embryonic stem cells to differentiate,

wherein the serum-free cell culture medium comprises a basal cell culture medium,

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

277. (New) A method for causing embryonic stem cells to differentiate in a serum-free cell culture medium comprising

- (a) contacting embryonic stem cells with a serum-free cell culture medium,
- (b) expanding the embryonic stem cells, and
- (c) adding a differentiation factor or changing culturing conditions thereby causing the embryonic stem cells to differentiate,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

278. (New) A method for obtaining embryonic stem cells in a serum-free cell culture medium comprising

(a) isolating embryonic stem cells from blastocysts, and

(b) expanding the embryonic stem cells in a serum-free cell culture medium,

wherein the serum-free cell culture medium comprises a basal cell culture medium,

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

279. (New) A method for obtaining embryonic stem cells in a serum-free cell culture medium comprising

(a) isolating embryonic stem cells from blastocysts, and

(b) expanding the embryonic stem cells in a serum-free cell culture medium,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

280. (New) A method for producing a recombinant protein in embryonic stem cells in a serum-free cell culture medium comprising

(a) obtaining a recombinant embryonic stem cell containing a nucleic acid molecule encoding a recombinant protein,

(b) contacting the recombinant embryonic stem cell with a serum-free cell culture medium,

(c) expanding the recombinant embryonic stem cell in the serum-free cell culture medium to form a population of recombinant embryonic stem cells, and

(d) isolating the recombinant protein from the population of recombinant embryonic stem cells or from the serum-free cell culture medium of (c),

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises an albumin, an albumin substitute, an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

281. (New) The method of claim 282 wherein the recombinant protein is isolated from the population of recombinant embryonic stem cells.

282. (New) The method of claim 282, wherein the recombinant protein is isolated from the serum-free cell culture medium of (c).